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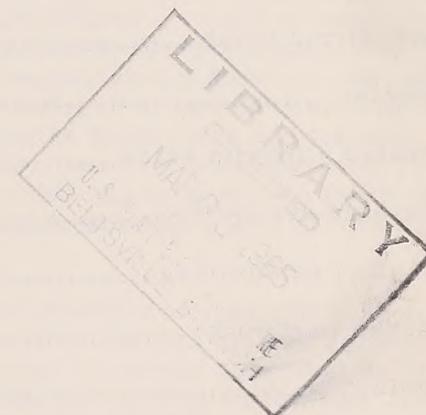
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## METABOLIC FATE OF HERBICIDES IN PLANTS



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# METABOLIC FATE OF HERBICIDES IN PLANTS

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## Introduction

The agronomic practice of controlling weeds by spray application of complex organic chemicals in small amounts is relatively new. As a consequence of extensive agronomic and physiological research with herbicidal chemicals, the stages of response through which a plant goes from the moment an herbicide is applied until death or recovery can be documented. Which species, and in some cases even which varieties, will not be greatly injured by a given treatment are well known. Although in most cases the mechanism by which an herbicide actually kills is not known with certainty, the effects produced upon such plant processes as differentiation, respiration, and photosynthesis can be described in some detail. It is further recognized that the various groups of chemicals used as herbicides differ markedly in their physiological effects and that the ambient environment may modify, and in some cases nullify, the effect of an herbicide.

Consideration of the chemical-plant interaction is incomplete without a complementary knowledge of what the plant does to the herbicide as a result of metabolic and chemical processes occurring in the plant. Studies of herbicide physiology and biochemistry must take into account not only what the variations from normal plant metabolism may be but also what modifications are induced in the structure of the herbicide molecule as a result of metabolic reactions in plant cells and tissues. The mechanism of action of an herbicide and its metabolic fate are interdependent. Alterations of chemical structure at the plant surface or within the tissues and their component cells may influence or determine the mechanism through which a lethal reaction is brought about.

Field application of an herbicide results in a distribution of the chemical on the soil surface or plant surface or both. That which strikes the soil may be partially degraded, adsorbed, or otherwise affected, but some of it may be absorbed by the plant roots. In the case of emerged plants a part of the spray will strike leaf and stem surfaces. Then it depends upon species, chemical, carrier and surfactants, ambient temperature and humidity, and physiological condition of the plant, what portion of the chemical striking the plant surface will be absorbed. That which remains on the plant surface will be retained by adsorption, lost by volatilization, absorbed by cuticle, complexed with cutin materials, washed off by rain, or be influenced by other factors. The fate of chemicals remaining on plant surfaces has received relatively little study. In general, the effort has been to attempt to increase contact with the plant

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surface in order to increase absorption. Qualitative and quantitative studies of the fate of herbicides remaining on plant surfaces offer a significant and extensive area for physiological research. Once absorbed the chemical may be rapidly translocated to other tissues where it may be degraded, accumulated, or complexed.

Concern for the possible presence of pesticides in edible products gives added reason for accelerated research into the metabolic fate of herbicides in crop plants. Knowledge is needed not only of the amounts of the parent herbicide remaining in the plant tissue at any given time after application but also of the quantity and nature of metabolites resulting from the metabolic activity of the plant upon the chemical.

It is the purpose of this review to present in some detail the published research relating to the metabolic fate of herbicides in plants.

### Symmetrical Triazines

The remarkable and useful selectivity of some of the symmetrical triazine herbicides for control of weeds in corn, sorghum, and other crops has led to extensive studies of the herbicidal potential of this group of chemicals. The recognition of relatively high triazine tolerance of corn and the pronounced effects upon photosynthesis have led to considerable interest and activity in the physiological action of these compounds. In the process, a metabolic degradation apparently unique to this group was discovered. A sizable literature has accumulated regarding the physiological activity of the triazines, their behavior in plants and soils, and their herbicidal efficacy. Only those papers dealing explicitly with the metabolic fate of these herbicides in plants will be considered here. The physiological aspects and metabolic fate of the triazines have been included in the reviews by Shaw and coworkers. (147),<sup>2</sup> Gysin (77), and in the 1963 reviews by Hilton, Jansen, and Hull, (90) and Freed and Montgomery (67). A comprehensive treatment of the triazines was presented by Gysin and Knüsli (78) who described in detail the early work done in Europe.

Earliest recognition of a degradation of 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine) is attributable to Roth (144) and his associates according to Gysin and Knüsli (78). Corn and wheat absorbed simazine-C<sup>14</sup> approximately equally from nutrient solution, but corn, which is resistant, contained much less simazine in the leaves than did wheat; this suggests that in corn an appreciable proportion of the chemical must have been metabolized. Roth incubated simazine with freshly pressed juice of young corn and wheat plants and found that in 100 hours most of the added triazine had been metabolized by the corn sap, but in wheat 90 percent of the simazine remained unchanged.

Early work in this country was reported in 1959 by Davis and coworkers (38) and Montgomery and Freed (119). Both groups noted a rapid absorption of triazines by corn. Montgomery and Freed found that the absorbed simazine and 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) were converted over a 4-week period to a chloroform-insoluble form. They interpreted these results as evidence for extensive metabolism of the triazines, since they were using ring-labeled triazine-C<sup>14</sup>. At least 9 percent of the absorbed simazine and 17 percent of the absorbed atrazine was degraded to C<sup>14</sup>O<sub>2</sub>. Davis and coworkers included cotton and cucumber with their study of corn: cucumber represented high susceptibility; cotton, intermediate susceptibility; and corn, high resistance. They noted that simazine, or a C<sup>14</sup>-degradation product, accumulated in the lysigenous glands of cotton. This has been substantiated by Hamilton and Moreland (83) for the closely related 2-chloro-4-diethylamino-6-isopropylamino-s-triazine (ipazine). Davis and his coworkers (38) did

<sup>2</sup>Numbers in parentheses refer to Literature Cited at end of this report.

not differentiate between the degradation products and simazine itself in their study of distribution of simazine-C<sup>14</sup>, but they did indicate that chloroform-solubles accounted for 50 percent of the absorbed radioactivity in cucumber leaves, 25 percent in cotton, and 5 percent in corn. Foy (56, 58, 60) and Foy and Castelfranco (61) have confirmed the ability of corn to degrade triazines. They found that not only were intact plants capable of degrading the herbicide, but also that coarsely chopped shoots and roots, plant macerates, and even particulate-free extracts of corn could degrade simazine (61). Only traces of C<sup>14</sup>O<sub>2</sub> evolution were noted.

In 1960 and 1961, a number of publications reported progress on triazine metabolism. The suggestion that an active principal of some sort was responsible for simazine degradation in corn was explored (32, 56, 78, 145). The active constituent was shown to be capable of converting simazine to 2-hydroxysimazine. Chemical and physical characterization of the active constituent in corn was attempted by Castelfranco and coworkers (32) and Roth and Knüsli (145). The pressed corn juice resistance factor was not sedimented by centrifugation at 100,000xg for 1 hour and was not destroyed by storage at freezing temperatures. In the crude extract, however, it was destroyed by boiling (32, 145). This active constituent was dialyzable, extractable by ether and ethyl acetate, soluble in 90 percent acetone, and was completely destroyed by ashing (32). Roth and Knüsli (145) suggested that the active principle might be associated with the phenol fraction of the plant extract. Upon isolation and recrystallization they obtained pink-colored crystals with a melting range of 159°-162° C, empirical formula equivalent to C<sub>9</sub>H<sub>9</sub>NO<sub>5</sub>, and R<sub>f</sub> of 0.83 in n-butanol:ethyl acetate: H<sub>2</sub>O (8:2:2), and an ultraviolet absorption maximum at 262.3 mu. They suggested that the isolated factor from corn juice was identical to 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one, a compound apparently identical to the cyclic hydroxamate which Hamilton and coworkers (81) isolated from etiolated corn seedlings and characterized chemically. Subsequently Roth and Knüsli (145) reacted this compound with simazine and found that, after 4 days incubation in aqueous buffer at pH 3.6 and room temperature, 85 percent of the simazine was converted to hydroxysimazine. The first clear-cut evidence of in vivo conversion of simazine to hydroxysimazine was presented by Hamilton and Moreland (82) who isolated the metabolite from corn seedlings.

Roth and Knüsli (145) recognized that discovery of the resistance factor in corn juice may not be the whole answer to triazine metabolism. Wheat and rye, which are susceptible to simazine, also contain benzoxazinone compounds. They suggested that other factors such as relative absorption rates and total capacity for triazine absorption may have been important.

Castelfranco and coworkers (32) speculated on the mechanism of conversion of simazine to hydroxysimazine. They suggested a nucleophilic attack on carbon 2 producing an unstable intermediate that reacts with water to form the hydroxy derivative. Citing the evidence for complete breakdown of simazine, they indicated that the possibility existed that hydroxysimazine may be further metabolized by pathways similar to that of pyrimidine in plants. Freed and coworkers (68) have proposed that hydroxysimazine may be metabolized further by oxidation to the keto form which could then readily be cleaved to produce CO<sub>2</sub>, basic amines, and other compounds, some of which may be incorporated into plant products by enzyme action. More recently Castelfranco and Brown (30) described a procedure for partial purification of the simazine-resistance factor of corn. They also found that pyridine and hydroxylamine reacted with simazine in a manner similar to the action of the corn extract. The similarity of pyridine and hydroxylamine to the hydroxamate reported by Roth and Knüsli was pointed out. However, the corn factor was 100 times as reactive in degrading simazine as was either pyridine or hydroxylamine.

Most attention has been paid to the 2-hydroxy derivative as a metabolite, but the work of Freed and coworkers (68), Montgomery and Freed (120), Ragab and McCollum

(138), Funderburk and Davis (69), and Hamilton and Moreland (83) showed that other metabolic products of triazines were also present. However, the preponderance of evidence suggests that the major early metabolite is an hydroxy derivative.

Foy (58) showed that the metabolic status of the roots may be of importance in triazine degradation. In studies using C<sup>14</sup>-labeled 2-chloro-4,6-bis(isopropylamino)-s-triazine (propazine) applied to sorghum, he found that when air was used as a flush gas considerable amounts of C<sup>14</sup>O<sub>2</sub> were evolved but when CO<sub>2</sub>-free air was used no measurable C<sup>14</sup>O<sub>2</sub> was produced. This does not, of course, preclude other forms of breakdown in the absence of CO<sub>2</sub>. Foy was unable to isolate an active resistance factor from sorghum even though this crop is highly resistant to triazines. Foy attributed this discrepancy to the possible existence of the resistance factor in sorghum as a glycoside, which would not have been extracted by his procedures.

It has been recognized that differences between species in susceptibility to triazines may be a quantitative function. Foy (58, 60) found that the susceptible oat does evolve C<sup>14</sup>O<sub>2</sub> from labeled triazines. However, if a methoxy group was substituted for the chlorine atom of propazine (prometone), then injury to sorghum resulted (60). Foy suggested that the methoxy-carbon bond was less susceptible to degradation than the chlorine-carbon bond.

Relatively little work has been done on the physiological factors affecting triazine metabolism. Numerous investigations have indicated the rapidity with which triazines are degraded. For instance Sheets (148) showed that within 24 hours after treatment a large percentage of C<sup>14</sup> from labeled simazine occurred in a chloroform-insoluble fraction in both oat and cotton roots. Most rapid metabolism was found with corn. Sheets noted further that degradation of simazine took place more rapidly in roots than shoots. This greater efficiency of roots than shoots was confirmed by Plaisted and Ryskiewich (135). These investigators also found that the rate of degradation in corn seedling roots was about the same in the light or in the dark, but that in corn leaves the production of hydroxysimazine was much less in the dark than in the light. They attributed this to reduced transpirational uptake and greater breakdown of simazine at night.

Although cotton shows tolerance to ipazine, this triazine is not readily degraded by cotton. Hamilton and Moreland (83) showed that ipazine itself accumulated particularly in the lysigenous glands and that only minor amounts of free hydroxyipazine could be found. There was some accumulation of an ethanol-insoluble, nondialyzable metabolite, and a chloroform-soluble, nondistillable material. The latter released hydroxyipazine upon hydrolysis with ethanolic hydrochloric acid. No C<sup>14</sup>O<sub>2</sub> evolution was detected from ipazine-C<sup>14</sup>.

Ragab and McCollum (138) do not agree with the previously demonstrated direct relationship between resistance to the triazines and rate or extent of degradation of the herbicide. Using sweet corn and cucumber seedlings treated with uniformly labeled simazine-C<sup>14</sup>, they demonstrated a more rapid C<sup>14</sup>O<sub>2</sub> evolution from the susceptible cucumber than the resistant corn plants. They attributed selectivity to rate of absorption and suggested that cucumber may absorb simazine at a greater rate than corn.

In preemergence studies with C<sup>14</sup>-ring-labeled atrazine applied to soil planted to species susceptible, intermediate, and resistant to the herbicide, Negi and coworkers (128) found that, in general, the ability to degrade atrazine was related to susceptibility but, among the susceptible species, beans metabolized much more atrazine than

did oats or soybeans. In order to assess whether the greater part of the degradation occurred in the soil or in the plants in their experiments, they analyzed the methanol-soluble materials in soil in which no seeds were planted; they found that less than 5 percent of the atrazine was metabolized in the 10-day period. Consequently, the majority of atrazine degradation was attributed to plant metabolism.

Hamilton (79) demonstrated that a corn mutant low in 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one exhibited less tolerance to atrazine than did a line of corn with normal content of the benzoxazinone. Furthermore, acetone extracts of the normal line converted 55 percent of added C<sup>14</sup>-simazine to C<sup>14</sup>-hydroxysimazine, but extracts of the low line metabolized only 22 percent of added simazine. Although the line of corn low in benzoxazinone was more susceptible to triazines than the normal corn, its benzoxazinone content was similar to that of wheat, which is much more susceptible. Hamilton postulated that detoxication of simazine to hydroxysimazine catalyzed by the benzoxazinone may not be a basic factor in tolerance and that other, possibly enzymatic, detoxication mechanisms may exist.

In a study of the ability of excised roots of several grass species to degrade triazine, Hamilton (80) found that tolerance to the herbicide was not related to the ability to metabolize 2-chloro-4,6-dialkylamino-*s*-triazines. Excised roots of corn, rye, and wheat degraded C<sup>14</sup>-simazine to C<sup>14</sup>-hydroxysimazine, but sorghum, oats, and barley did not. Determination of benzoxazinone content showed that those species that metabolized the triazine contained measurable amounts of benzoxazinone derivatives while those that did not convert simazine to hydroxysimazine contained no benzoxazinone. However, barley, oats, and sorghum roots contained detectable amounts of unknown water-soluble metabolites other than hydroxysimazine; this led Hamilton to suggest that other degradation mechanisms may have existed.

### Amino Triazole

As indicated by the number of literature citations included here, there has been considerable investigation of the metabolism of 3-amino-1,2,4-triazole. This herbicide has been called AT, amino triazole, and amitrole. Its metabolic fate in plants has previously been reviewed (67, 90, 147), and it is generally recognized that amitrole is readily metabolized by plants.

It was first suggested in 1955 by Rogers (141) that amitrole is converted in soybean plants to some other compound. He later identified the main water-soluble metabolite of amitrole in soybeans as a glucose adduct (142), and he indicated that amitrole was completely metabolized in this plant (143). Johnsongrass and Canada thistle also metabolize amitrole (142, 143) and, in addition to the glucose adduct, also produce other unknown metabolites, which are suggested to be attachments of amitrole to proteins, to carbohydrates other than glucose, or perhaps metal complexes (142). Rogers (143) also indicated that the metabolites may be produced nonenzymatically. Further support for the suggestion that amitrole is bound to protein was advanced by Aldrich and McLane (1) who noted the presence of nondissociable spots of bound amitrole on chromatograms of extracts of bean and wheat leaves. According to Castlefranco and Brown (31), labeled amitrole could add to proteins in the presence of a free radical generating system. Racusen (137) termed the amitrole metabolites that he detected in leaf tissue as compounds X and Y. He found that these compounds were stable to hot HCl and thus were not likely to be amitrole-protein complexes. In addition, Racusen concluded that X and Y were produced enzymatically in the leaves of pinto bean seedlings.

Yost and Williams (177) found that both corn and soybeans rapidly metabolized amitrole applied to the leaves. Although both species metabolized amitrole, they differed in that there was no marked accumulation of metabolites in corn. In soybeans

these workers found that the major metabolite increased to its maximum concentration 2 weeks after application. No attempt was made to identify the metabolite. In soil applications Palmer and Williams (131) noted that it took 20 to 25 days to reach a maximum metabolite concentration in soybeans. Two metabolites were found in soybeans, kidney beans, and pinto beans, and the authors concluded that they were the same as Racusen's X and Y.

Pastac (133) noted the formation of an abnormal, colorless complex in amitrole-treated plants but did not indicate whether the herbicide formed a part of this complex. Paul and Margoliash (134) were unable to detect any evidence for the formation of a compound between amitrole and peroxidase or any peroxide-peroxidase complexes.

Possible amitrole-amino acid complexes were reported by Carter (25) and Carter and Naylor (26, 29) and Massini (114) in 1959. When C<sup>14</sup>-labeled glycine and nonradioactive amitrole were fed to excised stem tips of Black Valentine beans, radioactivity was detected in an unknown, ninhydrin-sensitive compound, which was called compound 1 (26). Compound 1 was also formed when the tissue was fed amitrole-5-C<sup>14</sup> and unlabeled glycine. This unknown was not formed when glucose-U-C<sup>14</sup>, succinate-2,3-C<sup>14</sup>, or HC<sup>14</sup>O<sub>3</sub> were substituted for glycine-1-C<sup>14</sup>. Glycine-1-C<sup>14</sup>, glycine-2-C<sup>14</sup>, and serine-U-C<sup>14</sup> were all incorporated into compound 1. Acid hydrolysis of compound 1 produced amitrole but no glycine or serine; this suggested that compound 1 was a complex of amitrole and some fragment or derivative of the amino acids. In all, 14 amitrole derivatives were found in bean tissue (127). Compound 1 was the principal metabolic product in beans, alfalfa, honeysuckle, and maple. Most rapid metabolism occurred in beans, a highly susceptible plant (126). Carter and Naylor (28) and Naylor (126) attempted a characterization of their compound 1; they found that it was relatively stable to hydrolysis by acid or alkali. Partial hydrolysis was obtained only with 6 N HCl after autoclaving for 16 hours. The metabolite was not highly light sensitive and was found to act as a "zwitterion." It reacted more readily with Ehrlich's reagent than did the parent amitrole and gave a ninhydrin reaction. The authors concluded that the metabolite contained intact amitrole and probably contained several moles of amitrole per mole of compound 1. It was metabolically inert and as such was suggested to be a detoxication product of amitrole (28).

Massini (114) studied the metabolism of amitrole in French dwarf beans and attempted to characterize one of the metabolites, which he called ATX. Later Massini (116) showed that ATX was the principal product of the metabolism of amitrole in both French dwarf bean and tomato plants. He also found the presence of another metabolite of unknown composition. He was unable to show formation of any ATX by homogenates or extracts of tomato plants when incubated with amitrole although intact plants produced ATX within 24 hours after treatment. ATX was stable in the plant tissues and was translocated through phloem and xylem tissues. Massini (116) cited an unpublished X-ray diffraction study by Braun of the structure of ATX. It was found that ATX is  $\beta$  (3-amino-1,2,4-triazolyl-1)- $\alpha$ -alanine.

According to Herrett (86) amitrole was metabolized by both bindweed and Canada thistle but the rate of metabolism was more rapid in the relatively amitrole-resistant bindweed than in the susceptible Canada thistle. Two metabolites were detected by Herrett and Linck (88) in each species; they were named unknown I and unknown II. The latter metabolite was formed in greatest abundance and received the more detailed study. When both amitrole and glucose were infiltrated into bindweed leaves only unknown I was formed. Formation of the two unknowns was inhibited by 10<sup>-1</sup> M NaF. As indicated by Racusen (137) and Carter and Naylor (28), unknown II was found to be acid stable. Herrett and Linck suggested that their unknown II was similar to the amino acid-amitrole metabolites of Carter and Naylor (26), Massini (114), and Racusen (137).

The case for a glucose adduct of amitrole as originally suggested by Rogers (142) has been advanced by Fredrich and Gentile (62, 63, 64, 65, 66) and Gentile and Fredrich (73). These investigators have postulated the occurrence of an amine glucoside of amitrole formed by a secondary amine linkage between amitrole and glucose. The metabolic significance of this adduct has been questioned (90). Fredrich and Gentile (66) have indicated the formation of a phosphorylated derivative of the amine glucoside of amitrole, which they suggest could enter into the formation by plants of a triazolyltriose similar to the triazolylalanine suggested by Massini (114). They also postulate a complexing with protein or a reaction with phosphoserine to produce the triazolylalanine more directly.

Miller and Hall (117) treated seedling cotton leaves with amitrole- $^{14}\text{C}$  and detected the presence of two metabolites, which they called X and Y, within 4 days after treatment. Y was the more abundant metabolite. In mature cotton the same metabolites were found, and in the leaves Y was the abundant form while in the seeds X predominated. When amitrole was imbibed into cut leaves through the petiole, X was the main metabolite. They compared the  $R_f$  of X and Y with that of a number of amitrole adducts of organic acids, sugars, and metallic chlorides as well as ring cleavage and ring substitution products and various salts of amitrole and were unable to find any similarity. On the basis of their chromatographic evidence, they concluded that their X and Y are identical to those of Palmer and Williams (131) and Racusen (137).

A single reference by Freed and coworkers (68) deals with the possibility of a metabolic degradation of the amitrole ring with the resultant evolution of  $\text{CO}_2$ . Oats, an amitrole-resistant species, evolved  $\text{C}^{14}\text{O}_2$  from amitrole- $\text{C}^{14}$ -treated plants for an extended period of time, but only brief evolution of  $\text{C}^{14}\text{O}_2$  was observed from similarly treated barley, which is sensitive.

Hilton (89) found that the herbicidal activity of amitrole in several higher plant species was nullified by riboflavin, riboflavin-5-phosphate, and flavin adenine dinucleotide. He suggested that the rapid disappearance of amitrole from plant tissues might involve degradation of the herbicide through flavin-mediated systems. Castelfranco and his coworkers (31, 33) confirmed that amitrole was degraded by light in the presence of riboflavin and suggested that this breakdown occurred by a free radical mechanism. In their experiments this could presumably have occurred either in the plants (corn) or in the nutrient medium. Radioactivity from 3-amino-1,2,4-triazole-5- $\text{C}^{14}$  appeared in a number of compounds; this indicated extensive degradation. Approximately two-thirds of the radioactivity was lost, possibly as  $\text{CO}_2$  or formic acid (31). Amitrole can interact with free radical generating systems resulting in an activation of the chemical, and it was postulated that the activated amitrole may then act as an electrophile. Citing the work of Massini, they suggested that activated amitrole could attack carbon number 2 or 3 of alanine to form the triazolyl alanine derivative. This could occur with either the free or protein-bound amino acid. Free radical generating systems are known in plants and they could act to degrade amitrole (33).

Herrett and Bagley (87) have reported an additional amitrole metabolite in Canada thistle, which they have designated unknown III. This compound was more effective than amitrole in inducing discoloration and in inhibiting seedling growth. The authors suggested that it was an activation product of amitrole metabolism. Unknown III was apparently not an amine glucoside.

From the work described it is clear that there is not unanimous agreement on the identity of the major metabolites of amitrole. In addition, no attempts have been made to identify the possible minor metabolites. It is additionally evident that much work remains to be done in evaluating species and tissue differences in metabolism of amitrole under controlled environmental conditions.

## Maleic Hydrazide

Very little additional work has been published on the metabolism of maleic hydrazide since the topic was reviewed in 1960 (147). It was recognized at that time that maleic hydrazide was not readily or rapidly metabolized by plants. Although metabolism of herbicides has been reviewed again since then (67, 90), no new information on maleic hydrazide (MH) was cited.

Maleic hydrazide acts both as an herbicide and a plant-growth regulator. In the latter capacity the chemical acts to induce dormancy, as in the prevention of sprouting in potatoes, and to reduce growth of treated plants. MH is a slightly water-soluble, highmelting point, saltlike solid of very low vapor pressure. According to Smith and coworkers (151), it is highly resistant to hydrolysis by acid or alkali and not likely to be lost from a plant surface by volatilization or breakdown. Chemically, the compound is known as 1,2-dihydropyridazine-3,6-dione (147), or 6-hydroxy-3(2H) pyridazinone as proposed by Weller, Ball, and Sell (172).

Maleic hydrazide is rapidly absorbed by plant tissue, and absorption is accelerated by high humidities. Using the diethanolamine salt of MH, Smith and coworkers (151) found that 80 percent of the amount applied was absorbed from foliar applications to tomato plants under conditions of 100 percent relative humidity. Once absorbed, MH is not rapidly lost and may be either degraded or complexed with natural plant products. Residues may be detected in grass roots and in potatoes for as long as 8 months after treatment. Towers and coworkers (158) fed MH or MH-C<sup>14</sup> with or without glucose or glucose-C<sup>14</sup> to terminal segments of wheat leaves, and after short-term incubations of 12 hours they found that, using tagged MH, approximately 85 percent of the label remained as intact MH and about 15 percent appeared as a different radioactive spot, which they called U-1. A small amount (about 1 percent) of C<sup>14</sup> appeared as a spot, which they called U-2. When they used "cold" MH and glucose-C<sup>14</sup>, about 6 percent of the activity showed up as U-1. The C<sup>14</sup> in U-1 existed in the glucose portion when glucose-C<sup>14</sup> and MH were used and in the MH moiety when glucose and MH-C<sup>14</sup> were used. U-1 was hydrolyzed by emulsin indicating that this unknown was a  $\beta$ -glucoside of maleic hydrazide. Towers and coworkers (158) viewed this glucoside formation as a detoxication mechanism and suggested that U-1 existed as a disaccharide of glucose.

Parups, Hoffman, and Morley (132) have presented evidence that suggested that the hypothesis was incorrect that MH was degraded to either maleic or fumaric diamide (Isenberg, F. M. R., Ph.D. thesis, Pennsylvania State College, 1953). They also showed that tobacco plants were capable of splitting the benzoyl moiety from N-benzoyl-MH and the methyl groups from N-methylated-MH compounds. No evidence for direct degradation of MH was indicated.

Baker (13) has shown the formation of a complex of MH with plant constituents. He found that proteins could bind MH and that the bond strength was greater for the diethanolamine than the potassium salt. Homogenates of plants treated with MH-C<sup>14</sup> and precipitated with ammonium sulfate were labeled. This radioactivity was not readily lost by overnight dialysis against water or unlabeled MH; hence, Baker considered that forces binding MH and protein were physical rather than chemical.

## Chlorinated Phenoxyacetic and Phenoxypropionic Acids

The metabolic fate of 2,4-dichlorophenoxyacetic acid (2,4-D) and closely related herbicides has been studied by a number of investigators and the subject has been

reviewed four times since 1959 (7, 67, 90, 147). Despite the fact that these compounds have received more study than other types of herbicides, the details of their metabolic fate in plants remain unclear. There is no agreement on the extent, rate, or pathway of degradation of the phenoxy herbicides. However, numerous papers do indicate that these compounds undergo a variety of reactions in plant tissue. For purposes of clarity, the subject will be treated here by types of possible metabolic products or disposition, rather than as an historical exposition.

### Metabolism by Decarboxylation

One of the most widely recognized fates of 2,4-D has been that of its decarboxylation by plant tissue (5, 6, 9, 12, 23, 24, 39, 40, 45, 92, 102, 103, 109, 110, 121, 150, 164, 165, 166, 167, 169, 170, 175). It was suggested in 1950 by Holley and coworkers (92) that the loss of 2,4-D in red kidney bean plants 7 days after treatment may have been caused by release of  $C^{14}O_2$  from the carboxyl-labeled herbicide. That 2,4-D can be decarboxylated has been amply demonstrated since then, but the extent and importance of this degradation remain unclear. Weintraub and his associates (165) clearly demonstrated decarboxylation by bean seedlings treated with 2,4-D labeled in both the carboxyl- and methylene-carbons. They found that the plants evolved  $C^{14}O_2$  three times as rapidly from the carboxyl as from the methylene position. Fang and coworkers (45), using methylene-labeled  $C^{14}$ , attempted to quantitate the loss; they noted that in 3 days 17.5 percent of the 2,4-D applied to the midrib of the primary leaf of bean plants was lost as  $C^{14}O_2$ . Weintraub and coworkers (166) noted that evolution of  $C^{14}O_2$  from plants treated with 2,4-D continued at a rather low rate for several days from carboxyl- or methylene-labeled herbicide but when the number 1 ring carbon was labeled, no  $C^{14}O_2$  was produced. Using resistant corn plants, Weintraub and coworkers (167) found no marked difference in decarboxylation of 2,4-D from that produced by beans. As a result they suggested that resistance to 2,4-D was not associated with rapidity of decarboxylation of the herbicide. They found that  $C^{14}O_2$  evolution from 2,4-D labeled in the carboxyl position was about 1 percent per day of the total amount applied to corn leaves. Bach and Fellig (9, 12), working with beans, supported Weintraub's suggestion that metabolism of 2,4-D involved relatively little decarboxylation. Basler (18) compared the rate of decarboxylation of 2,4-D and 2,4,5-T in excised leaves of several woody species. He found that maximum decarboxylation amounted to about 1 percent for 2,4-D and a trace for 2,4,5-T.

Contrary to Weintraub's work, others have found marked differences in species and varietal ability to decarboxylate 2,4-D. Edgerton (39) and Edgerton and Hoffman (40) showed that  $C^{14}O_2$  evolution from radioactive 2,4-D was much more rapid from leaves of the resistant McIntosh apples than from leaves of susceptible Winesap and Stayman varieties. Intermediate between them were Baldwin and Northern Spy. Further evidence for wide differences in ability to decarboxylate 2,4-D and related compounds by closely related varieties and species has been advanced by Luckwill and Lloyd-Jones (109, 110). They compared the ability of the sensitive black currant and the resistant red currant to decarboxylate carboxyl- and methylene-tagged 2,4-D-C<sup>14</sup>. In 1 week, 50 percent of the carboxyl and 20 percent of the methylene C<sup>14</sup> were evolved as  $C^{14}O_2$  in red currant, but only 2 percent of either form of tagged 2,4-D in black currant (109). Their work with apples confirmed that of Edgerton in that detached 2,4-D resistant Cox apple leaves decarboxylated 57 percent of the applied 2,4-D in 4 days whereas only 2 percent was decarboxylated in the same time by the susceptible Bramley's seedling variety. Again in strawberry resistant varieties decarboxylated 2,4-D more extensively than did susceptible varieties.

The more rapid  $C^{14}O_2$  evolution from carboxyl- than methylene-labeled 2,4-D led Weintraub and coworkers (165) to suggest that acetic acid derived from the 2,4-D side chain entered into the tricarboxylic acid cycle prior to  $CO_2$  production. Canny and Markus (24) showed that  $C^{14}O_2$  was produced from tissues treated with 2,4-D- $C^{14}$ . They agreed that this was not the result of direct decarboxylation but rather that the acetate radical was lost intact and that the radioactive  $CO_2$  was evolved from subsequent metabolism of the acetate moiety. They found greater labeling of  $CO_2$  by roots than shoots and attributed this to a greater ability of the roots to degrade 2,4-D. This indirect decarboxylation idea was supported by Leafe (102, 103) who studied the metabolism of 2-methyl,4-chlorophenoxyacetic acid (MCPA) and 2-(2-methyl,4-chlorophenoxy) propionic acid (2-(MCPP)). Ten days after treatment of Gallium aparine with these chemicals most of the applied MCPA- $C^{14}$  was metabolized, but nearly all of the 2-(MCPP) remained unaltered. Of the metabolized MCPA about 7 percent appeared as respiratory  $C^{14}O_2$ . Leafe concluded that MCPA was detoxified by first splitting off the 2-carbon side chain rather than by a direct decarboxylation, and in the case of 2-(MCPP) that the presence of an  $\alpha$  methyl group blocked the loss of the side chain.

Weeds, too, differ in their ability to decarboxylate 2,4-D as shown by Slife and coworkers (150) and Williams and coworkers (175). Jimsonweed and burcucumber did not differ significantly in their ability to decarboxylate 2,4-D, but neither species evolved very much  $C^{14}O_2$  (175). Using wildcucumber (Sicyos angulatus), which is resistant to 2,4-D but susceptible to 2,4,5-T, Slife and coworkers found that  $C^{14}O_2$  evolution was 10 times greater from plants treated with 2,4-D than from those treated with 2,4,5-T (150).

Contrary to previous ideas, Morgan and Hall (121) found that cotton decarboxylated 2,4-D- $C^{14}$  several times faster than did sorghum. They concluded that side chain decarboxylation was not an important mechanism of resistance to 2,4-D.

### Metabolism by Hydrolysis of Esters and Amides

Wheat coleoptiles metabolized N-2,4,5-trichlorophenoxyacetyl-l-glutamic acid and N-2,4,5-trichlorophenoxyacetyl-d-glutamic acid as indicated by the appearance of 2,4,5-T (22). Sudi and coworkers (153) also showed that N-substituted methyl, ethyl, propyl, isopropyl, and butyl amides of 2,4-D and 2,4,5-T and the corresponding propionic acid derivatives were degraded to the parent acids.

More definitive information is available on the hydrolysis of phenoxy esters. Using 2,4-D- $C^{14}$  tagged in the carboxyl group or in the alcohol chain, Crafts (35) found that the alcohol portion of an ester largely stayed in the treated leaf whereas the acid residue moved basipetally. Morré and Rogers (122) used a bioassay in which roots of cucumber seedlings were inhibited by 2,4-D acid over a narrow concentration range in which the octyl ester of 2,4-D had no effect. This provided a method for detection of free acid formed from hydrolysis of the octyl ester. They were able to detect a rate of hydrolysis adequate to account for complete hydrolysis in the intact plant. Kidney bean epicotyl curvature tests also established the hydrolysis of octyl and propylene glycol butyl ether esters of 2,4-D.

Jooste (97) and Jooste and Moreland (98) isolated and partially characterized enzymes from wheat seeds and cucumber, corn, and soybean seedlings, which were capable of hydrolyzing phenoxy esters. These esterases (97), or carboxylic ester

hydrolases (98), were located in the epidermis, cambium, and phloem; this indicated that the esters can be hydrolyzed at or near the site of absorption. The rate of hydrolysis was greater for unchlorinated than for chlorinated phenoxyacetic acid esters and greater for monochloro than for dichloro derivatives. Szabo (156) showed that hydrolysis of butoxyethanol and propylene glycol butyl ether esters of 2,4-D occurred both on the surface and within the tissues of corn and bean leaves. In addition, bean plant homogenates produced 2,4-D acid from the esters of 2,4-D.

## Metabolites

Sugar complexes.--Jaworski and Butts (95) found three unknown metabolites of 2,4-D in 80 percent alcohol extracts of bean stems from plants treated with 2,4-D-C<sup>14</sup> with the label in either the carboxyl or methylene position. Their unknown 1 had an R<sub>f</sub> of 0.55 to 0.59 in water-saturated phenol and 0.29-0.32 in butanol:propionic acid:water. This 2,4-D metabolite was formed in relatively large amounts and was quite stable. They suggested that unknown 1 may be a glycoside with 2,4-D as the aglycone. This suggestion was challenged by Bach and Fellig (10), because their major metabolite moved at the same R<sub>f</sub> (0.5) in the same solvent system used by Jaworski and Butts but did not contain any carbohydrate. However, Klämbt (100) found that a glucose ester of 2,4-D was formed during incubation of wheat coleoptiles with the acid. This glucose derivative occurred in smaller amounts than a 2,4-D-amino acid complex. Leafe (102) suspected that MCPA was split at the ether linkage, which resulted in a phenol residue. Because it occurred in the water-soluble fraction, the suggestion was made that the phenol would conjugate with a sugar to form a glycoside or similar sugar complex. Direct evidence of such a compound was not presented, however.

Amino acid complexes.--The earliest suggestion that an amino acid derivative of 2,4-D formed in plant tissue treated with 2,4-D was proposed by Andreae and Good (4). Pea epicotyls incubated for 24 hours with 2,4-D produced small amounts of 2,4-dichlorophenoxyacetyl aspartic acid. However, Zenk (179) was unable to detect a conjugation of 2,4-D with glycine under conditions where indoleacetic acid formed the glycine conjugate. Klämbt (100) confirmed the metabolic formation of 2,4-D-aspartic acid in wheat coleoptile cylinders. Good and Andreae (75) found little or no 2,4-D-aspartic acid.

Bach (8, 12) found 10 distinct ninhydrin-positive metabolites of 2,4-D in bean stems treated with 2,4-D-1-C<sup>14</sup> that were identified as  $\alpha$ -amino acid derivatives. He suggested that some of the major metabolites previously described were probably complex mixtures of amino acid amides of some unknown acid metabolites of 2,4-D. Leafe's (102) work with Galium aparine also indicated the presence of a minor metabolite of MCPA and 2-(MCPP), which he thought might be an amino acid conjugate.

Protein complexes.--The hypothesis that 2,4-D could complex with plant protein was introduced by Fang (41) and Butts and Fang (23). The two metabolites (unknowns 1 and 3) that they found were indicated to be protein complexes. These protein-2,4-D complexes were found in pea and tomato plants (41) as well as in corn and wheat (23). Protein complexes were also formed by 2,4,5-T, 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), and 2-chlorophenoxyacetic acid. Upon hydrolysis these protein complexes produced aspartic acid, glutamic acid, glycine, serine, threonine, alanine, tyrosine, methionine, phenylalanine, valine, leucine, and isoleucine. Both major protein-2,4-D metabolites produced comparable amounts of the same amino acids, which suggests a similar origin. Based on their studies of the metabolic fate of 2,4-D in tick bean, Canny and Markus (24) believed that the major pathway for metabolism of the herbicide was an initial binding to protein for 4 or 5 days followed by extensive degradation of an unspecified nature. On the other hand, Leafe (102, 103) indicated that such 2,4-D complexes were probably of minor importance.

Unknown complexes.-- That 2,4-D and its relatives may exist in plants as complexes was implied or inferred as early as 1952 (95). In many cases the type of complex suggested has been based on varying amounts of evidence or even subjective considerations. Fang and Butts's (42) unknowns 1 and 3 are acid hydrolyzable complexes derived from corn and wheat plants harvested 3 days or more after 2,4-D treatment. Further work suggested that unknown 1, which had an  $R_f$  of 0.44 in n-butanol: propionic acid: water, was formed independently of photosynthesis (96). Later Fang (41) indicated that both unknown 1 and 3 were protein complexes.

Bach and Fellig (9) found that about half of the 2,4-D absorbed by bean stem sections was bound in the tissue, and they noted that it was hydrolyzable by refluxing in 2 N HCL for 2 hours (10). Further evidence for a bound form of 2,4-D (as much as 30 percent of that applied) was cited for currants (109) as well as apple and strawberry leaves (110). In these cases, too, weak hydrolytic conditions would not release free 2,4-D. Bach and Fellig's (11) bean stem callus tissue contained an ethanol-soluble complex of 2,4-D moving to  $R_f$  0.5 in butanol: propionic acid: water (8:5.6:12). Others have indicated the presence of unidentified and uncharacterized complexes (164) or have surmised that a labile complex acts as the translocation form of 2,4-D (111).

In 1963 Morgan and Hall (121) found a 2,4-D complex in cotton, which they considered to be the same as Fang's unknown 1 in beans. The complex that they isolated from sorghum was considered to be similar to Fang's unknown 3.

Metabolites other than complexes.--As early as 1950, Holley and coworkers (92) noted the presence of a water-soluble fraction in red kidney bean plants treated with 2,4-D that contained C<sup>14</sup> from the carbon-labeled herbicide. Later Holley (91) suggested that at least part of the metabolite was a 3-, 5-, or 6-hydroxy 2,4-D. At the same time Weintraub and coworkers (170) found a small amount of 2,4-D metabolite in bean plants that either volatilized or slowly decomposed. Generally, the radioactive carbon from 2,4-D was found to enter a variety of natural plant products rather rapidly in beans. The extent of metabolism was thought to be low in dormant buds of cherry (169) but nevertheless distinct (168). Leaper and Bishop (104) postulated that plants may metabolize 2,4-D to a quinone and then to chloromaleic acid. No evidence has been presented to support this hypothesis.

Jaworski and coworkers (96) found small amounts of unidentified metabolites of 2,4-D in beans at  $R_f$  0.22 to 0.28, 0.36 to 0.38, and 0.65 to 0.68 in n-butanol: propionic acid: water (8:5.6:12). In currants, 5 to 10 percent of the 2,4-D absorbed by detached leaves was converted to inactive, water-soluble metabolites (109) and in strawberries the phenol content of the leaves increased as 2,4-D was degraded (110). Presumably phenol was derived from decarboxylation and demethylation of 2,4-D. Metabolites of 2,4-D from bean stem sections, with  $R_f$  0.5 in butanol: propionic acid: water (8:5.6:12), were considered to be detoxication products of 2,4-D (11). Some of these were shown to have additional phenolic and aliphatic hydroxyl groups attached, and it was suggested that a lengthening of the acetic side chain may have taken place (8). In addition to the previously mentioned glucose ester and aspartic acid derivative of 2,4-D, Klämbt (100) found three other metabolites that were not identified or characterized. The uncertainty of identification of 2,4-D metabolites and complexes was emphasized by Slife and coworkers (150), who found three fractions in wild and cultivated cucumbers treated with carboxyl-labeled 2,4-D-C<sup>14</sup> and 2,4,5-T-C<sup>14</sup>. No identification was attempted. Morton and Meyer (123) found that 80 percent of the 2,4,5-T-C<sup>14</sup> applied to mesquite seedlings was metabolized to some unidentified product within 25 hours. Similar rapid production of unidentified metabolites of 2,4-D-C<sup>14</sup> were reported in ironweed (Vernonia baldwinii) by Linscott and McCarty (108).

Wilcox (173) and Wilcox and coworkers (174) have demonstrated the occurrence of a metabolite of phenoxyacetic acid in roots of oats, barley, and corn that contained an hydroxyl group in the ring. Chromatographic and spectrophotometric evidence indicated that the metabolite was 4-hydroxyphenoxyacetic acid. However, no hydroxylated derivatives were detected when roots were exposed to 4-chloro-, 2,4-dichloro-, or 2,4,5-trichlorophenoxyacetic acid.

### Rate of Metabolism

Physiological factors in the fate of 2,4-D have generally received only incidental attention. The major effort has gone into attempting to determine the presence and possible composition of metabolites in plant tissues. However, the various data that are available may serve as guides for further study.

The preponderance of evidence suggests that metabolism of 2,4-D and related compounds is relatively rapid (6, 8, 22, 23, 24, 41, 42, 45, 84, 85, 91, 92, 95, 108, 109, 110, 111, 123, 167, 174). In dormant tissue, metabolism of the herbicide is much slower (160), 168, 169). Weintraub and associates' work (168, 169) with dormant buds of cherry seedlings showed that this relatively inactive tissue was still capable of metabolizing 2,4-D. As much as half of the 2,4-D absorbed by dormant cherry seedlings from bud treatments was metabolized during the winter (168). Little other work has been done on dormant tissues. One report from Russia indicated that decomposition of 2,4-D in dormant Canada thistle roots was very slow (160).

As pointed out by Ashton (6), the 2,4-D content of any plant or tissue will be a resultant of the absorption, translocation, and metabolism of the herbicide. If absorption and translocation exceed degradation, then the 2,4-D content can build up and the metabolism of the chemical will not be so apparent. In red kidney bean, Ashton found that this occurred during the first 24 hours but between 24 and 72 hours after treatment the balance shifted to favor metabolism, which resulted in a decrease in 2,4-D content. It would seem most important to consider these factors in any investigation where absorption and metabolism are occurring simultaneously.

Relatively low rates of  $C^{14}O_2$  evolution by plants treated with 2,4-D-C<sup>14</sup> have been noted in the case of cotton and sorghum (121), beans (166), cucumber (150), as well as jimsonweed, burcucumber, and cocklebur (175). On the other hand, appreciable  $C^{14}O_2$  evolution was found in beans (45, 92), detached apple, strawberry (110), and red currant leaves (109). Luckwill and Lloyd-Jones (110) have reported some of the highest rates of decarboxylation, but they also found very low rates by a number of plants such as plum, pear, rose, and a variety of weed species.

Of greater interest are the reports of differential rates of metabolism between resistant and susceptible species. The data of Luckwill and Lloyd-Jones again are the most striking. The 2,4-D resistant apple variety Cox decarboxylated 2,4-D in detached leaves to the extent of 57 percent of the absorbed herbicide, but in the susceptible Bramley's seedling only 2 percent of the 2,4-D was decarboxylated in the same time period. In these cases, and with detached leaves of strawberry, they found the most rapid metabolism initially with rapidly decreasing rates thereafter (110). This was attributed to a decreasing amount of 2,4-D substrate rather than reduced capacity for metabolism. Mature leaves of strawberry metabolized 2,4-D twice as fast as young leaves.

Luckwill and Lloyd-Jones (110) further indicated that oxidation of the methylene carbon occurred at less than half the rate of the carboxyl-carbon. But Canny and Markus (24) showed that  $C^{14}O_2$  evolution by tick bean was equal from treatment with either 2,4-D-1-C<sup>14</sup> or 2,4-D-2-C<sup>14</sup>. Later Morgan and Hall (121) calculated that the efficiency of release of  $C^{14}O_2$  in cotton was greatest from the methylene carbon of

tagged 2,4-D. The latter calculation was based on the likelihood that degradation does not proceed by progressive decarboxylation of the acetic side chain but rather by splitting the side chain at the ether linkage.

Canny and Markus (24) found a greater rate of  $\text{C}^{14}\text{O}_2$  production from 2,4-D by roots than shoots of tick bean, and they suggested that this was evidence for a greater capacity for degradation of the herbicide in roots. Based on discrepancies in rates of metabolism between cut tissue and intact plants, they suggested that cut tissue was less efficient in degrading 2,4-D. In addition, they proposed that rate of breakdown was modified by the initial binding of 2,4-D to root proteins during the first 4 or 5 days after treatment. Subsequently, the rate of metabolism was rapid.

The differences in rate of metabolism of 2,4-D by cotton and sorghum were striking and surprising. Morgan and Hall (121) found that the rate of side chain degradation by cotton seedlings was from 5 to 10 times greater than that of sorghum seedlings. Cotton produced a 2,4-D metabolite (complex 1) that differed markedly from that produced by sorghum (complex 3). In cotton, complex 1 did not exceed the concentration of 2,4-D for 3 days while in sorghum, complex 3 concentration exceeded that of free 2,4-D in less than 1 day. Their data suggested a lack of correlation between susceptibility and ability to metabolize 2,4-D and related herbicides, but others have noted a direct correlation between resistance and degree of activity in metabolizing these chemicals. The data of Luckwill and Lloyd-Jones (110) and of Slife and coworkers (150) cited earlier are pertinent. Fang (41) noted far more rapid breakdown of 2,4-D in pea plants than in tomato plants.

Most investigators noted that 2,4-D was at least partially metabolized within a week. Hay and Thimann (85) studied the disappearance of 2,4-D in bean seedlings over a 48 hour period; he found that the recovery of parent compound decreased from 94 percent 6 hours after treatment to 44 percent at the end of the period. A 50-microgram application of 2,4-D to bean seedlings disappeared almost completely and about half of the absorbed compound was metabolized in just 1 day (84). Light was also implicated in capacity to metabolize the herbicide. They found a more rapid loss of 2,4-D in the light than in the dark.

Holley and coworkers (91, 92) noted rates of 2,4-D degradation in beans very similar to those of Hay and Thimann. In mesquite, Morton and Meyer (123) found a relatively rapid metabolism of 2,4,5-T. Of that absorbed by fully expanded leaves, 80 percent was changed in 25 hours. In addition, the environment of the plants prior to treatment affected the amount of metabolism of the herbicides. They determined that 2,4,5-T metabolism was at a maximum in the range from  $70^{\circ}$  to  $85^{\circ}$  F., at a lower rate at  $100^{\circ}$  F., and completely inhibited at  $50^{\circ}$  F.

Wilcox and coworkers (174) have formalized mathematically the rate of metabolism of phenoxyacetic acid. The specific velocity constant for the formation of 4-hydroxyphenoxyacetic acid was determined as follows:

$$kt = \ln \frac{a}{a-x}$$

where:

t = time interval in hours  
a = initial concentration  
x = decrease in concentration  
during time interval t

The k value reported for barley was  $5.12 \times 10^{-4} \text{ hr}^{-1}$ .

## Miscellaneous Factors in Metabolism

Physical factors, such as binding of 2,4-D to inactive materials in the plant cells, exert distinct influences upon the extent and type of metabolism possible. Leopold (106) measured the adsorption of 2,4-D and related compounds onto charcoal and steam-killed leaves; he found that there was an influence of chlorination upon strength of adsorption. Most strongly adsorbed was 2,4,5-T, next 2,4-D, and least 4-chlorophenoxyacetic acid. Ultraviolet light can cause degradation of 2,4-D, primarily through its effect upon the phenolic moiety of the herbicide (19). This could be of slight significance in the fate of 2,4-D on the plant surface. Gibberellic acid does not change the rate of breakdown of 2,4-D in bean plants (6, 17).

Work by Crosby (37), using microcoulometric gas chromatography, indicated that the ether-soluble component of extracts of bean plants treated with 2,4-D was the parent herbicide. In plants treated with 2,4-D, the major portion of the chlorinated material extracted 4 days after treatment was 2,4-D. Of the two major water-soluble, ether-insoluble metabolites detected, the major one appeared to be a readily hydrolyzable metabolite, which Crosby suggested might be a sugar ester. The other metabolite may have involved a basic structural change but probably existed as a sugar derivative also. He suggested that many of the previously reported metabolites were artifacts.

## Phenoxybutyric Acids

Based upon their work with various plant-growth regulators, Synerholm and Zimmerman (155) suggested that omega phenoxybutyric acids may be degraded in plant tissues by the process of  $\beta$ -oxidation. This oxidation would result in the conversion of the biologically inactive butyric derivative to the highly active acetic form. In general, they predicted that omega(2,4-dichlorophenoxy)alkanecarboxylic acids having an odd number of side-chain methylene groups would yield, by  $\beta$ -oxidation, the active 2,4-dichlorophenoxyacetic acid, whereas those having an even number of methylene groups would produce 2,4-dichlorophenol when acted upon by appropriate plant tissue. This work was based on studies with tomato plants, and it was amply confirmed in flax roots in England by Fawcett, Ingram, and Wain (49), who also extended the studies to include the omega-(4-chlorophenoxy)alkanecarboxylic acids. Here again the alternation of activity with number of side chain methylene groups was observed and attributed to  $\beta$ -oxidation. In addition to the species mentioned, Wain and Wightman (163) demonstrated an alternation of activity in wheat cylinder and pea curvature tests. In the omega-(2,4,5-trichlorophenoxy)alkanecarboxylic acid series, they found that while the odd-numbered methylene derivatives were active in wheat, only the acetic derivative was active in pea and tomato tissue. This raised the possibility that the ability of the  $\beta$ -oxidation enzyme complex to degrade the growth-regulating chemical may depend upon the source of the enzyme, and that whether or not a given plant will degrade the side chain may depend upon the nature and position of the nuclear substituents on the phenoxy compound. This work has been reviewed by Fawcett and coworkers (50) in 1955 and by Wain (162) in 1961.

These investigations led Wain (161) to postulate in 1955 that a basis for selective weed control might exist based upon the enzyme makeup of the plant and its ability to degrade the aryloxy acid. He found that celery plants sprayed with a series of the omega-(2,4-dichlorophenoxy)- or omega-(2-methyl,4-chlorophenoxy)-alkanecarboxylic acids were injured only by the acetic derivative but that nettle was killed by the acetic, butyric, and caproic derivatives. This indicated a  $\beta$ -oxidation of the applied compounds to the acetic form by nettle but not by celery. Similarly, it was found that legumes such as red and white clovers did not degrade the higher homologs whereas important weeds infesting these crops could  $\beta$ -oxidize the compounds and were killed.

Freed and his coworkers (68) prepared an active acetone powder of peas that contained an enzyme system capable of  $\beta$ -oxidizing 4-(2,4-dichlorophenoxy)butyric acid (4-(2,4-DB)). They coupled the oxidation of the growth-regulator to reduction of TPN and followed the subsequent reduction of tetrazolium by TPNH photometrically. Using radioactive 4-(2,4-dichlorophenoxy) butyric acid in the presence of the pea acetone powder, they were able to demonstrate the production of a number of metabolites, none of which were identified.

Glastonbury and coworkers (74) found a very rapid breakdown of the butyl ester of 4-(2,4-DB) in seedling lucerne, but they noted that degradation of 4-(2,4-DB) was much slower in plants treated with the ester than with the sodium salt. They indicated the importance of environmental factors in determining the extent of persistence or rate of loss of the parent 4-(2,4-DB).

A method for the analysis of 4-(2,4-DB) and its metabolite 2,4-D has been reported. Using this technique, Gutenmann and Lisk (76) were able to detect 0.2 p.p.m. of 4-(2,4-DB) and 0.1 p.p.m. of 2,4-D. They showed a progressive decrease of 4-(2,4-DB) from over 100 p.p.m. early in the birdsfoot-trefoil growing season to almost none in August. The 2,4-D content increased from less than 1 p.p.m. in mid-June to about 5 p.p.m. in July and then decreased to minimal levels in early August. Fertig and coworkers (51) found that formation of 2,4-D from 4-(2,4-DB) occurred to about the same extent in birdsfoot-trefoil and timothy. They were also able to demonstrate that this degradation took place in sterile pea plants; thus, the possibility that micro-organism metabolism may be solely responsible for the conversion of 4-(2,4-DB) to 2,4-D was eliminated. Linscott (107) noted differences in the rate of metabolism among several forage species. Metabolism, or loss of 4-(2,4-DB), was most rapid in birdsfoot-trefoil, followed by ladino clover and alfalfa, and the slowest rate of degradation occurred in red clover. Most rapid metabolism occurred immediately after treatment. Linscott also found that variations in rate of degradation or disappearance of the herbicide were caused by environmental conditions. Heavy rainfall greatly decreased the rate of 4-(2,4-DB) metabolism. Whether simple washing away or some more complex mechanism was involved are unknown.

A complete understanding of 4-(2,4-DB) degradation rests only partially in an understanding of  $\beta$ -oxidation. The metabolite, 2,4-D, also may be degraded by many plants, and it is probably these products that Freed and coworkers (68) found in their studies.  $\beta$ -oxidation of the omega phenoxy alkanecarboxylic acids is a classic in the literature of herbicide metabolism. It should be kept in mind, however, that much remains to be learned of the details of the enzymatic mechanism in various species. Evaluation and interpretation of the largely unknown effects of environment upon the metabolic fate of this class of herbicides continue to be a challenge to physiological research.

## Carbamates

As indicated in the earlier review by Shaw and coworkers (147) and later reviews by Hilton and coworkers (90) and Freed and Montgomery (67), carbamates apparently do not retain their identity for long in treated plants. Several investigators have noted the lack of a residue (70, 71, 129); and some have attributed this to a metabolic disposition of one type or another by the plant (70, 140).

Relatively little work has been done with the carbamates to investigate their possible metabolic products. Analytical techniques for the herbicides isopropyl N-phenyl carbamate (IPC) and isopropyl N-(3-chlorophenyl) carbamate (CIPC) are readily available (70, 71), and it is now generally accepted that these early-discovered and important carbamates leave no detectable residue in those crop plants that have been investigated. Few studies have been reported on the identification of possible

metabolites. According to Baskakov (15), sunflowers metabolize IPC much more rapidly than do oats and destruction of the herbicide is essentially complete within 4 days after treatment. He presumed that the carbamate was hydrolyzed to aniline followed by "oxido-reductive" metabolism, and he attributed the sensitivity of germinating cereal grains to the inability of their roots to metabolize IPC. Earlier work of Baskakov and Zemskaya (16) suggested that IPC may undergo an oxidation to N-hydroxy IPC. Zemskaya and Rakitin (178) employed the same test species and also found a very rapid destruction of IPC. Applied to the leaves, IPC was metabolized rapidly in both sunflower and oats; but, when root applications were made, metabolic degradation was much slower in oat than in sunflower roots. No attempt was made to identify the metabolic products.

Studies on the degradation of the thiolcarbamate herbicides have been conducted by Fang and his coworkers (44, 46, 47, 48). They found that ethyl-N,N-di-n-propyl-thiolcarbamate (EPTC) was absorbed from a soil application by a variety of horticultural crops but that less than 3 percent of the total amount absorbed remained as free EPTC (46). EPTC content was measured as S<sup>35</sup> from EPTC-S<sup>35</sup>; thus, the possibility that this 3 percent was unmetabolized is not assured. More recently a detailed study was conducted of the absorption and translocation of EPTC-S<sup>35</sup> after preemergence application to peas, corn, radish, carrot, and other horticultural crops (47). The radioisotope was rapidly absorbed and translocated to the shoots. Absorption occurred over a long period of time and accumulated chiefly in the roots of beans, peas, and corn and in the leaves of radishes and carrots. No analysis of the possible metabolic products was attempted. EPTC has been found to accumulate in growing stem tips and root tips after application to the leaves; but, when applied to the roots, the distribution was more general and EPTC was present in mature tissues as well (176). In seeds and seedlings, Fang and Yu (48) found that there was a decrease in EPTC-S<sup>35</sup> only in those species that are resistant to the herbicide. They indicated that the sulfur atom was oxidized to the sulfate form, which was incorporated into sulfur-containing amino compounds.

Fang and George (44) also studied a closely related herbicide, propyl-1-C<sup>14</sup>-(N, N-ethyl, n-butyl) thiolcarbamate as a soil treatment. Mung beans, which are resistant, and wheat, which is susceptible, were grown in the treated soil and the absorption of C<sup>14</sup> and the rate of breakdown of the thiolcarbamate-C<sup>14</sup> were estimated. Breakdown was rapid in mung beans, but wheat did not degrade the herbicide. Some of the C<sup>14</sup> was recovered as respiratory C<sup>14</sup>O<sub>2</sub>, and some was found in other plant constituents.

Foy (57) studied 4-chloro-2-butynyl-N-(3-chlorophenyl) carbamate (barban) translocation; he found that there were no apparent differences in the amount of barban-C<sup>14</sup> absorbed or translocated by barley, oats, and wild oats. Leaf treatments of these species with ring-labeled barban-C<sup>14</sup> resulted in no accumulation of the radioisotope in the grain at harvesttime. Presumably barban is readily metabolized.

More detailed study of the metabolic fate of barban in plants has been reported by Hopkins (93) and Riden and Hopkins (139, 140). Hopkins found a water-soluble metabolic product of barban in wheat plants 3 days after spraying. This "X compound" was also found in barley, peas, sugar beets, safflower, flax, and wild oats. "X compound" was found to occur within 24 hours after spraying but gradually decreased after 2 to 4 days. The rate of barban metabolism was more rapid at high temperatures, but less "X compound" accumulated. Hopkins suggested that "X compound" contained the carbamate linkage and at least the number one carbon atom of the butynyl group. The metabolite was apparently not an activation product, because "X-compound" was found to be inactive against wild oats (93). The lack of residue of barban in barley, peas, sugar beets, and wheat at maturity was confirmed by Riden and Hopkins (139); they did not obtain residues even with excessive rates of application.

Riden and Hopkins (140) further investigated Hopkins's 3-chloroaniline-containing "X-compound" and noted that it was formed not only by barban but by other analogs as well. Because "X-compound" contained 3-chloroaniline, it seems certain that the plant does not degrade the benzene ring initially. They evaluated the possibility of the formation of an N-hydroxy derivative, but they found that the water solubility was too low to be "X-compound." Neither did alteration of the triple bond or chloromethyl group produce a compound identical with "X-compound." From this evidence they concluded that the unknown water-soluble substance was not a direct metabolite of barban. Therefore, it was most likely a combination or addition product with some naturally occurring plant component.

Jaworski (94) studied the metabolism of 2-chloroallyl-N,N-diethyldithiocarbamate (CDEC) in resistant 4-week-old cabbage sets planted in soil treated with 6 pounds per acre of CDEC-C<sup>14</sup> labeled in the 2-position of the 2-chloroallyl radical. Three weeks after treatment the plants were harvested, and 80 percent aqueous acetone extracts of the homogenates were made. Most of the radioactivity (83.3 percent) occurred in the acetone extract. Fractionation of the extracted material on ion exchange columns resulted in a general distribution of the radioactivity among all the fractions. Jaworski suggested that CDEC and its 2-chloroallyl moiety were extensively degraded. Radioactivity associated with the major component was shown to correspond to lactic acid by chromatography. He further suggested that lactic acid could be formed directly from the 2-chloroallyl portion of CDEC.

The precise nature of the metabolites of the carbamate compounds has not been discovered, and the modifying influence of environment upon degradation of carbamates by higher plants will require intensive study.

### Chlorinated Aliphatic Acids

Of primary concern in this group of herbicides are trichloroacetic acid (TCA) and 2,2-dichloropropionic acid (dalapon) and their salts. When the subject was first reviewed (147) it was indicated in general that these herbicides were either not metabolized in plants or at least that they disappeared only very slowly. Chlorinated aliphatic acid metabolism has been reviewed by Freed and Montgomery (67), Hilton and coworkers (90), and Leasure (105).

Using a colorimetric method for analysis, Tibbitts and Holm (157) determined the TCA content of young snapbeans, millet, peas, and red beets 5 days after a soil treatment with TCA at approximately 85 pounds per acre (12 mg./4-inch pot). Other than species, not physiological variables were introduced. Their method did not distinguish between TCA and its possible degradation products; nor did it exclude the possibility of immobilization as a complex with other plant materials, and they recognized the possibility of such alteration. No data on fate of TCA were presented. Thus the bulk of the information on this grass killer rests with a single paper. Blanchard (20) studied the fate of TCA in corn and pea plants exposed to a nutrient solution containing 34 p.p.m. TCA-2-C<sup>14</sup> for 3 days. The presence of labeled carbon at harvest was shown, and chromatographic analysis showed that the radioactivity was all in the form of the original TCA molecule. No physiological variables were introduced.

Thus the lack of metabolic alteration of the TCA molecule is based almost entirely on results published in a single report (20). Conclusive as this report appears, it deals only with two species treated at a single rate of application at a single stage of growth and grown under a single constant set of light, temperature, and moisture conditions as well as a single very short (8 hour) day length. It would seem that a more complete study is needed in any thorough and painstaking program of research into the fate of TCA in plants.

That dalapon is absorbed, translocated, and accumulated largely as the original chemical in plants has been shown repeatedly (3, 21, 36, 52, 54, 55, 59, 136, 152). Absorption of dalapon by either the roots or leaves of cotton resulted in extensive distribution throughout the plant (36, 52, 54, 55, 152) with accumulation being greatest in regions of high metabolic activity (36, 55, 152). Similar accumulation tendencies have been noted for sugar beets (2, 3), barley (36), sorghum (54, 55), wheat (55), and corn (59).

The most intensive series of papers on dalapon metabolism is that by Foy (52, 53, 54, 55, 59) who used autoradiography, extraction and fractionation, counting, and paper chromatographic techniques to analyze for the herbicide and its possible metabolic products.

Small amounts of radioactivity could be detected in the water of guttation of sorghum treated with 2,2-dichloropropionic acid-C<sup>136</sup>, but none could be detected when treated with Na-2,2-dichloropropionate-2-C<sup>14</sup> (54). It was suggested that the radioactivity possibly was due to small amounts of inorganic C<sup>136</sup> removed from the herbicide in long-term experiments. Loss was also found to occur as leakage from the roots of cotton (54, 152) and sorghum (54) when there was an unfavorable ionic balance in the nutrient medium. The excreted radioactive material was chromatographically indistinguishable from authentic dalapon-2-C<sup>14</sup> (54).

In an intensive study of dalapon metabolism in cotton, Foy (55) incubated tissue homogenates with dalapon-2-C<sup>14</sup> and dalapon-C<sup>136</sup> for short periods; he was unable to find any change in the herbicide. When applied to intact cotton plants, no metabolic product was apparent for the first week after treatment. Later, a small amount of nonextractable radioactivity appeared in both cotton and sorghum; this suggests that some of the dalapon had been incorporated metabolically into the plant constituents. Quantitatively it accounted for 1 percent or less of the applied chemical. This non-extractable residue in cotton was interpreted differently by Smith and Dyer (152). They suggested that this was actually occluded or trapped, but unaltered, dalapon. Continuous extraction of the residue gradually decreased the content of this component. The slow metabolism of dalapon was emphasized by the finding (59) that in cotton and wheat the herbicide was carried over from one generation to the next.

Most studies with dalapon have been of relatively short duration. Blanchard and coworkers (21) agreed that dalapon resisted metabolism based on their studies with soybeans and corn in which no metabolic products were found 4 days after treatment when the herbicide was applied to roots or foliage.

One must exercise care in long-term studies, such as that of Schreiber (146) on birdsfoot trefoil, to interpret decline of herbicide content properly. As Schreiber indicated, what seems to be a loss of the chemical may actually be a dilution due to growth.

It has been suggested (112) that differences in the rate of translocation of dalapon in the light and in the dark could be attributed to the formation of a chemical or physical combination of the herbicide with a light-formed natural metabolite. According to this hypothesis, a prolonged period of darkness would be required to break down the herbicide-metabolite combination and thus result in increased translocation of dalapon.

### Benzoic Acid Derivatives

Very little research has been done on the metabolic fate of benzoic acid and its derivatives, and it has received only brief mention in current reviews (67, 90). However, a little information from a few sources will at least indicate the status of research on these compounds.

Benzoic acid itself is readily metabolized by plant tissue. In pea epicotyl tissue the chemical was rapidly absorbed and metabolized (4). About three-fourths of the absorbed radioactivity from carboxyl-labeled benzoic acid-C<sup>14</sup> did not accumulate in the tissue, and most of that which did accumulate appeared predominantly as benzoylaspartic acid. Only a trace of free benzoic acid existed along with small amounts of other radioactive materials. Since this work of Andreae and Good (4) utilized carboxyl-labeled carbon only, it cannot necessarily be deduced that the entire benzene ring has been lost from the plant tissue.

Klämbt (99) also used carboxyl-labeled benzoic acid-C<sup>14</sup> and noted the formation of an amino acid combination: benzoylasparagine. A considerable amount of radioactivity was found in benzoic acid-glucose ester form. As the incubation time increased, the amount of label in the benzoylasparagine increased. Six other undetermined benzoic acid metabolites were noted. He also found that benzoic acid was converted to salicylic acid and its glycoside in Helianthus hypocotyls (101).

Little additional work has been published on benzoic acid metabolism in plants. Pallas (130) referred briefly to the rapid metabolism of the compound and to the fact that it may be translocated without necessarily being metabolized first.

Among the herbicidal benzoic acid derivatives, there have been no published reports concerning the metabolism by plants of 2-methoxy-3,6-dichlorobenzoic acid, 2,4-dichlorophenoxyethyl benzoate, polychlorobenzoic acid, and 2,3,5,6-tetrachlorobenzoic acid. The only two which have received limited study are 3-amino-2,5-dichlorobenzoic acid (amiben) (14, 68) and 2,3,6-trichlorobenzoic acid (106, 113).

Freed and coworkers (68) conducted nutrient culture experiments in which carboxyl-labeled amiben-C<sup>14</sup> was supplied through the roots to soybeans. Baker and Warren (14) applied carboxyl-labeled amiben-C<sup>14</sup> to both roots and shoots of the relatively resistant squash and susceptible cucumber. They concluded that oxidative breakdown of amiben occurred chiefly in the roots. Baker and Warren (14) noted two chromatographically separable radioactive spots in the shoots of both cucumber and squash and three or more metabolites in the roots. In the shoots, most of the radioactivity was in the spot identical with amiben; whereas, in the roots most of the radioactivity was limited to an unidentified spot. The unidentified metabolites did not react to tests for amino acids or sugars.

More work is needed to establish clearly the number and nature of amiben metabolites in a variety of plants of varying susceptibility and to determine quantitatively the importance of decarboxylation versus esterification or other metabolic action in the fate of this herbicide. The response to environmental changes is completely unexplored, and no headway has been made into determination of metabolic pathways involved.

In dead leaf tissue, 2,3,6-trichlorobenzoic acid is less strongly absorbed than 2,4-D; and within the benzoic acid series, adsorption increases with the number of ring-substituted chlorine atoms (106). Whether this is related to the metabolic fate in living plant tissues for the benzoics is not known.

A related herbicide, 2,6-dichlorobenzonitrile, has been considered briefly by Massini (115). Bean plants exposed to vapors of the chemical for 6 days metabolized about half of the total absorbed. The presence of a metabolite was established chromatographically, but no identification was attempted.

Amiben is subject to measurable alteration in aqueous solution when exposed to ultraviolet or sunlight. Sheets (149) has shown a disappearance of amiben and the occurrence of two major changes in the molecule by chromatographic means. The extent of ultraviolet or sunlight destruction on a plant surface has not been determined.

## Chloro Alkyl Acetamides

Work by Hannah has been cited by Gysin and Knüsli (78) which suggests that 2-chloro-N,N-diallylacetamide (CDAA) may be degraded in corn in a manner similar to that of the triazines. The chlorine atom of CDAA may be hydrolyzed to form the hydroxy derivative, which can be further hydrolyzed to form glycolic acid and diallylamine. Both of these could be further metabolized or complexed by plants. Shaw and coworkers (147) cited Wangerin, who indicated that corn and soybean seedlings metabolize CDAA completely within 4 or 5 days after emerging through soil treated with the chemical. Susceptible plants lack the ability to hydrolyze CDAA.

Jaworski (94) found that CDAA was rapidly metabolized by corn seedlings following a preemergence application of the chemical. Using a sensitive ryegrass germination bioassay, he found that CDAA was almost completely metabolized within 4 days after emergence. Chromatographic analysis of extracts of corn seedlings treated pre-emergence with carbonyl-labeled CDAA-C<sup>14</sup> showed a metabolite which coincided with glycolic acid in 4 day old plants. There was no hydroxylated CDAA or chloroacetic acid present. Further chromatographic study indicated that the major early metabolite of CDAA was glycolic acid. More than half of the radioactive CDAA absorbed by the roots was evolved as C<sup>14</sup>O<sub>2</sub>. Using CDAA labeled in the 2-carbon position of the allylic radical, Jaworski found a random distribution of radioactivity throughout the plant constituents. Appreciable quantities of C<sup>14</sup>O<sub>2</sub> were evolved. In the species studied degradation was rapid and complete. There was no evidence for the existence of stable intermediate.

## Phenylurea Herbicides

Despite the appreciable interest in this very effective group of weed-killing compounds, only a few reports of metabolic fate are available. Only a single paper was cited in Shaw (147) review in 1959, and very little more has been reported since then (67, 72, 90).

That 3-(*p*-chlorophenyl)-1,1-dimethyl urea (monuron) gradually disappears from treated plant tissues has been recognized since 1955 (43, 124, 125). Muzik and co-workers (124, 125) concluded that the decrease in concentration of monuron in velvet-bean plants grown in nutrient cultures containing 100 p.p.m. of the herbicide was due to a metabolic process. Fang and his coworkers (43) studied the fate of monuron in bean plants. Using a combination of paper chromatography and radioisotope detection, they found an unknown monuron complex. As the monuron concentration decreased with time, the concentration of the complex increased. Van Oorschot (159) found an inactivation of monuron in plantain to which 2x10<sup>-5</sup> M concentration of the chemical was applied.

Except for the statement by Crafts (34) that monuron apparently is not split by urease, no studies of enzyme systems capable of metabolizing the phenylureas have been published.

Welker and Holm (171) studied the metabolic fate of monuron in seedlings of Abutilon theophrasti that were treated with the C<sup>14</sup>-labeled herbicide. Chromatographic separation of extracts showed that in 2- to 3-day-old seedlings there were three radioactive substances while in 7-day-old seedlings there were only two. The

metabolites differed from monuron in their absorption spectrum and in the fact that they fluoresced in UV light while monuron absorbs UV light.

The work of Sweetser (154) showed that monuron may complex with flavine mononucleotide (FMN) in Chlorella and that this complex upon illumination inactivated monuron. In this photoinactivation there was at least one major reaction product and a number of lesser materials formed. The major product appeared to have several monuron molecules associated with it, and a portion of the riboflavin molecule may also be incorporated in it. However, this is not necessarily the way in which monuron is metabolized in higher plants.

The recent work of Geissbühler and coworkers (72) on the metabolism of N'-(4-chlorophenoxy)-phenyl-NN-dimethylurea is a significant contribution to the knowledge of the fate of the urea herbicides in plants. They found that leaves and especially roots of corn and bean were able to demethylate the herbicide to N'-(4-chlorophenoxy)-phenyl-N-methylurea, N'-(4-chlorophenoxy)-phenylurea, and (4-chlorophenoxy)-aniline. Two other unknown metabolites were detected. The authors suggested that degradation of N'-(4-chlorophenoxy)-phenyl-NN-dimethylurea was primarily by demethylation and that most active breakdown occurred in the roots.

### Cyanamide

The only studies on metabolism of calcium cyanamide in plants reported in the United States are those of Miller and Hall (118). They cite work by Hofmann and co-workers in Germany who extracted an enzyme from soybeans (cyanamidase) that degraded cyanamide. They also found that when treated with cyanamide-C<sup>14</sup>, barley and corn evolved C<sup>14</sup>O<sub>2</sub>. Hofmann's group demonstrated that 2 to 3 days after treatment with cyanamide oats, wheat, barley, and rye converted part of the herbicide into dicyandiamide. In addition, the concentration of certain amino acids increased; this suggested incorporation of the degradation products of cyanamide. Miller and Hall also referred to a review by Latzko that indicated that Rathsack was able to find not only dicyandiamide but also guanidine and guanyl compounds as cyanamide decomposition products.

In their own investigations, Miller and Hall (118) worked with the metabolism of cyanamide in cotton. Using cyanamide-C<sup>14</sup> and paper chromatographic techniques, they found a number of metabolic products of the herbicide, and suggested that the pathway was through urea to other products. In plants in which the cyanamide was injected directly into the bolls, no cyanamide remained after 9 days, but other C<sup>14</sup>-labeled materials were detected. In intact cotton plants metabolism was rapid with no free cyanamide being detectable in leaf extracts 8 hours after treatment. Three major metabolites were detectable within 4 hours after treatment. One of the major products was chromatographically indistinguishable from urea. Another was apparently alanine or a closely related compound.

### Discussion and Conclusions

The group of chemicals that are classified as herbicides is an extremely heterogeneous one, both from the standpoint of the chemical structure of its constituent members and of the biological activity resulting from their use. Differential

selectivity varies from virtually none at one extreme to nearly single species selectivity at the other. The examples of variation in reaction by crop and weed plants to herbicides and to a given chemical under different environmental conditions are numerous. Although a significant proportion of the differential reactions are based on physical factors both of plant structure and surface characteristics, an even greater proportion must be attributed to processes occurring within the crop or weed plant that modify the reactivity of the plant and that literally alter the chemical configuration of the herbicide molecule, which successfully penetrates the various barriers to absorption. Depending upon the particular herbicide and species concerned, the chemical may be subject to early translocation from the site of absorption to other plant organs via phloem or xylem transport either as the intact molecule or in some modified form. Varying amounts of the chemical may be retained at or near the absorption site and here again the possibility for retention as the unchanged molecule or an altered or complexed form exists. It is conceivable that metabolic or chemical alteration of the herbicide may occur en route during the translocation process, particularly where phloem transport is concerned. Weed-killing chemicals are frequently translocated to regions of relatively high metabolic activity, and consequently they can be influenced by a number of active or potentially active enzymatic processes. A herbicide that has been partially metabolized or has undergone complexing with native plant products could be further metabolized at centers of high activity, such as stem tips, developing leaves and buds, root tips, and young branch roots.

The early research in the metabolic fate of herbicides in plants has been directed toward assessment of the disappearance or persistence of the chemical in species of known selectivity. In many cases this has resulted in the discovery of one or more metabolites that may in turn disappear rapidly or persist for varying periods of time. In addition to the ability of the plant to degrade or complex the chemical, the accumulation of the herbicide or its metabolites depends upon the extent to which a source of unreacted weed killer remains available, such as by slow versus rapid absorption through the leaves or roots, or by repeated chemical treatment.

Major attention has been given to the phenoxy herbicides, the *s*-triazines, and amino triazole. A few reports of the metabolic fate of maleic hydrazide, the herbicidal carbamates, benzoic acid derivatives, and the phenylureas have been cited. In no case can the course, direction, rate, pathways, or complete products of metabolism be documented with certainty. Most accessible to analysis has been the degradation or modification of a side chain or ring substituent in a number of herbicides. The  $\beta$ -oxidation of chlorinated phenoxybutyric acids, ester hydrolysis and  $\text{CO}_2$  production from the phenoxyacetic acid acetate moiety, and hydroxylation of the 2-position on the *s*-triazine ring have been repeatedly demonstrated, and considerable confidence can be placed in these data, but it should be reemphasized that these reactions are undoubtedly only initial steps in further metabolic sequences. In the case of  $\text{CO}_2$  evolution from 2,4-D and related compounds, there is uncertainty whether the reaction is by direct decarboxylation or whether the ether linkage is first split releasing the acetate group which is subsequently oxidized to  $\text{CO}_2$ . Critical evaluation of these possibilities is needed.

It is significant to note that not only does the concentration of the absorbed herbicide in the plant increase rapidly and then decrease at a usually slower rate, but also that the major metabolites follow a similar trend in many cases, frequently with a noticeable lag phase. The disappearance of these readily detectable metabolites with time signifies clearly a further disposition of the foreign chemical; and the appearance of a number of minor metabolites,  $\text{CO}_2$  evolution, and labeling of natural plant products indicate rather extensive secondary degradation of the initially detoxified chemical. This area of research is one in which much remains to be done. Where the initial metabolic fate is one of activation rather than inactivation, as is true for 4(2,4-DB), the ultimate question of the disposition of the resultant 2,4-D remains. Similar problems exist where the initial fate of a chemical involves a complexing with carbohydrate, as has been postulated for amino triazole and maleic hydrazide; with

protein, as has been claimed for a number of herbicides; with amino acids, as indicated for 2,4-D, amino triazole, and benzoic acid derivatives; and with flavin mononucleotide, as has been reported for monuron. Subsequent degradation is probable, but the pathways and intermediates are not well known. Metabolites have been found in plants treated with carbamates but their identity is not known in all cases, and carbamate degradation is so rapid that analysis of intermediates is difficult. At the opposite extreme, the chlorinated aliphatic acid herbicides are highly resistant to degradation in higher plants. The factors limiting their metabolism are unknown.

A wide variety of techniques has been utilized in determining the metabolic fate of herbicidal chemicals in plants. The high sensitivity of bioassay methods have served well in both qualitative and quantitative analysis of residues and accumulation of unaltered or non-detoxified chemicals, but in the usual case where the metabolite showed no activity upon the test organism, bioassay seemed to be of little value. However, the method should always be considered where biological entities sensitive to herbicid metabolites can be found. This may require extensive evaluation of untried plant materials and microorganisms. Furthermore, analysis of the behavior or degradation of a given herbicide in organisms other than the plant of primary interest may provide valuable clues to what is happening in the plant.

The use of radioisotope-labeled herbicides has proven invaluable in recent research on pesticide metabolism and can be considered to be an even more significant tool in the future. Primary interest has centered around use of C<sup>14</sup>, but, where applicable, Cl<sup>36</sup> and S<sup>35</sup> have been useful. Double-labeling of the herbicide, tritium labeling, and tagging suspected intermediates should prove helpful in the future. In addition to the widely used techniques of Geiger and proportional counting and their adaptations for very low level counting, liquid scintillation counting of properly separated components of extracts of treated plants will provide high efficiency. In addition to these, greater use of the isotope dilution technique is needed and, where applicable, neutron activation analysis. In addition to, and in combination with, radioisotope usage, continued utilization of paper, column, thin layer, and gas chromatography will be profitable. With these techniques it is possible to separate and detect the presence of metabolites at low levels, and by cochromatography with suspected intermediates rapid progress can frequently be made in tentative identification of degradation products. Possible metabolites might be synthesized, appropriately labeled, and the subsequent fate of the substance followed in sequence and in comparison with the parent herbicide. A promising technique where herbicide complexes are anticipated is to feed labeled or unlabeled herbicide to the plant along with labeled or unlabeled components of normal plant metabolism and analyze the resultant complexes. This has been used successfully, and further evaluation of this technique is warranted.

Although the use of radioisotope-labeled herbicides and suspected intermediates will contribute greatly to the research program, there will be a need to take advantage of many other chemical and physical techniques. In those instances where complexing of the herbicide with protein occurs, electrophoretic analysis could yield valuable information. Ultraviolet, visible, and infrared spectrophotometry as well as spectrofluorometry may be used to identify chemical groups and atoms in degradation products. It is possible that mass spectrometry, atomic absorption spectrometry, and nuclear magnetic resonance spectrometry may be employed in definitive determinations of the chemical nature of metabolites. "Wet" chemical analytical procedures will lend themselves to analysis of herbicide complexes and metabolites when sufficient material is available for determination. Histochemical procedures followed by light or electron microscopy and autoradiography could aid in cellular and subcellular localization of degradation products.

Important as metabolite identification may be, a cooperative effort of the analytical chemist with the biochemist and plant physiologist will be necessary for a coherent establishment of a balance sheet of herbicide disposition by plants. For adequate understanding of the metabolic processes involved in the degradation of the

herbicide, the biochemist will need to evaluate possible pathways, both enzymatic and chemical, by which the chemical is ultimately degraded. This will involve detailed study of enzyme identification, localization, kinetics, and characteristics as well as the possible effect of the parent herbicide upon the ability of the enzyme to degrade it. Although well known in microbial physiology and biochemistry, the induction of enzymes has not been intensively studied in higher plants. Perhaps the time course of herbicide metabolism in many plants may depend upon and be explained by induction of suitable degradative enzymes. This possibility seems worthy of further investigation; it could have a bearing upon the metabolism of repeated applications of an herbicide in resistant plants.

Thus far the more purely physiological phases of herbicide fate in higher plants have received little attention. That variations in atmospheric and soil environment influence plant growth and metabolism markedly are widely recognized, but these effects have not yet received the attention they deserve in herbicide metabolism. Through the use of the analytical techniques previously mentioned, evaluation of the various permutations and combinations of day length, light intensity, soil and air temperature, soil moisture, humidity, and nutritional status of the plant in a wide variety of species, varying from resistant to susceptible, to a given chemical upon its metabolic fate is needed. The environmental conditions prior to and after treatment may markedly influence the ability of the plant to translocate and degrade a herbicide. In order that this research be meaningful, the environmental factors must be controlled to a high degree in suitable plant growth chambers.

The possibility that the herbicide may be modified prior to or during absorption by the plant must be taken into account. Chemical and physical effects at the root, leaf, or stem surface may significantly influence the structure of the absorbed molecule and will govern the rate and extent of absorption. In addition, the solvent and wetting agent employed in applying the chemical can affect the fate of an herbicide at the plant surface. Environmental effects such as temperature, rainfall, wind velocity, dew deposition, and relative humidity cause variations in the loss of chemical before absorption and alter distribution on the shoot. Losses by volatilization are highly significant in many cases and will require consideration in evaluation of the balance sheet of herbicide disposition.

Evidence is mounting that herbicides applied to the soil may be adsorbed on soil particles; this limits root absorption or modifies the rate of absorption, which, in turn, may influence the plant's ability to detoxify the chemical. Microbial metabolism of the herbicide may so alter the chemical that what is actually absorbed by the plant root is significantly different from the parent compound. Research on the behavior of herbicides in soils will need to be taken into serious consideration in those instances where plant metabolism of chemicals applied to the soil is concerned.

In modern chemical weed control, increasing use is being made of combinations of herbicides applied simultaneously to plant or soil surfaces. Such multiple herbicide applications may include component chemicals of similar or widely dissimilar characteristics. In addition to all the other factors previously discussed for single herbicide metabolism, it will be necessary to consider: (a) the possibility of direct chemical effects between the two or more component herbicides, (b) the effect of one chemical upon the course of metabolism of the other, (c) alterations produced in normal plant metabolism by one of the herbicides that may affect the degradation of the other, and (d) differential rates of absorption and translocation. Other possible complicating factors will undoubtedly affect the metabolic fate of herbicides in multiple pesticide applications.

It is apparent that in research devoted to an understanding of the fate of chemicals applied to plants in extremely small amounts, refinements of techniques and innovation will take precedence over routine analysis. The importance of such research is manifest in the significance of herbicide usage in agricultural production.

Efficiency, selectivity, and safety of herbicide usage can be greatly enhanced by high-quality research in the field of plant metabolism of chemicals used in weed control. The magnitude and scope of the problems discussed here require team effort, and the inherent difficulties make the challenge to imaginative scientific endeavor all the greater.

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